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Die-Away Kinetics of Aerosolized Bacteria from Sprinkler Application of Wastewater

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A methodology for estimating, under field conditions, the microbial die-away constant (λ) is presented. This constant may be used in predicting the aerosolized pathogenic microorganism concentrations downwind from a wastewater spray or aeration site by means of modified atmospheric diffusion equations. The mean λ of *Escherichia coli* for very early morning runs was 8.8×10^{-3} s^{-1}, and that for afternoon runs was 6.6×10^{-2} s^{-1}.

The need to predict the concentration of aerosolized pathogenic microorganisms downwind from a wastewater spray or aeration site has grown in importance with the renewed impetus given to wastewater reuse and disposal by land treatment.

In many of these applications, wastewater effluent is sprayed under pressure with 0.1 to 1% of the liquid being aerosolized (7, 21). In wastewater treatment processes involving aeration, aerosols can be formed containing concentrations of enteric microorganisms 100 times or more greater than that detected in the ambient liquid (5). In a similar manner, enteric microorganisms from wastewater disposed of into the sea are aerosolized at significantly higher concentrations than found in the source water and can be carried airborne to nearby shore areas (3).

Air monitoring of such aerosolized microorganisms has led to the detection of enteric microorganisms as much as 1,200 m downwind from a sewage treatment plant (1) and 350 m downwind from a sewage sprinkler irrigation field (14). Similarly, pathogenic enteric viruses have been detected by us 100 m downwind from sewage spray irrigation nozzles.

The potential health risks associated with the dispersion of aerosolized pathogens from such sources have still not been fully determined, but some presumptive evidence indicates that increased enteric disease among nearby residents may be associated with such wastewater irrigation practices (13).

To properly design and evaluate wastewater land application facilities involving sprinkler irrigation techniques or other wastewater treatment or disposal works which may be associated with the aerosolizing of pathogenic microorganisms, it is essential to develop and validate a model for predicting downwind microbial concentrations based upon concepts of atmospheric diffusion and microbial die-away as a function of various meteorological and climatological parameters.

Airborne microorganisms are transported downwind by atmospheric diffusion and ultimately are deposited on the ground. During the atmospheric transport, microbial die-away occurs in the air. The die-away is a function of several factors, including cellular physiological characteristics (2), relative humidity (10, 11), temperature (9), oxygen concentration (8), light (13, 20), and air pollutants (16, 18). The die-away rate varies with the quantity and quality of these factors.

Because it is impractical to carry out long-term monitoring near all sources of live aerosols, it is proposed to use a mathematical model for the prediction of aerosol concentration in the environment as a criterion of potential health hazards. Such a model, despite its deficiencies, may allow the calculation of microorganism concentration at sites lacking monitoring equipment and may also enable optimal placing of aerosol sampling and monitoring equipment.

It is our objective to apply and validate a relatively simple model for estimating the potential concentration of live microorganisms at the breathing zone level downwind from a continuous point source. The first essential steps have been the development of a methodology for determining the bacterial die-away constant, lambda (λ), under field conditions as a function of key environmental parameters, and to validate the atmospheric diffusion model under the specific conditions of spray irrigation. These two matters are the objective of this study.

An atmospheric diffusion model. A relatively simple model may be used for estimating the potential concentration of live microorganisms at ground level, downwind from a contin-

uous point source, when given the following factors: (i) the microbial die-away rate; (ii) wind velocity; (iii) atmospheric stability class; (iv) height of the source above ground level; (v) concentration of microorganisms in source; and (vi) efficiency of aerosolization of source equipment.

Modeling efforts to date have used different atmospheric dispersion equations (4, 19, 25). These models are generally adequate in their ability to predict aerosol behavior and plume distribution in relation to the source parameters and meteorological conditions. However, they are inadequate in that they fail to take into account the loss in aerosol strength caused by biological death. The Gaussian plume model allows the atmospheric dispersion prediction for inert pollutants [$\chi(x,y,z)$] released from a point source, when the height of the source (H) and the meteorological conditions are known (25). The concentration of the pollutant for a receptor at a distance x,y from the source and at a height z above ground is given by equation 1.

$$\chi(x,y,z) = \frac{Q}{2\pi\sigma_y\sigma_z\bar{u}} \exp\left[-\frac{1}{2}\left(\frac{y}{\sigma_y}\right)^2\right]\left\{\exp\left[-\frac{1}{2}\left(\frac{z-H}{\sigma_y}\right)^2\right] + \left[-\frac{1}{2}\left(\frac{z+H}{\sigma_z}\right)^2\right]\right\} \quad (1)$$

where χ is concentration of the pollutant in the air (i.e., the number of particles per cubic meter of air) at a distance (x,y,z) from the source; Q is rate of release (i.e., the number of particles emitted from the source per second); $\bar{u}$ is the mean wind velocity (meters per second); and σ_y and σ_z are the diffusion coefficients of the material in the plume in the y and z direction (meters); these are functions of meteorological conditions and of the downwind distance from the source.

The height of the source (H) and the downwind, crosswind, and vertical coordinates (x, y, and z, respectively) are expressed in meters. In this model, the following assumptions are made: (i) the plume has a Gaussian distribution in the vertical and horizontal planes; (ii) the particles are completely reflected from the ground; (iii) the source emits at a constant rate; (iv) wind velocity and direction are constant for a given time and place; (v) the ground surface is flat; (vi) the aerosol particles are smaller than 20 μm and therefore gravitational settling is negligible; and (vii) the relative velocity between the wind and the source is negligible, as is the diffusion downwind.

For a ground level receptor ($z = 0$), equation (1) becomes:

$$\chi(x,y,0) = \frac{Q}{\pi\sigma_y\sigma_z u} \exp\left[-\left(\frac{y^2}{2\sigma_{y^2}} + \frac{H^2}{2\sigma_{z^2}}\right)\right] \quad (2)$$

and for a ground level source ($H = 0$) and for a receptor along the center line of the plume, equation 2 becomes:

$$\chi(x,0,0) = \frac{Q}{\pi\sigma_y\sigma_z u} \quad (3)$$

Modification of the diffusion model, considering biological death of microorganisms. Several modeling approaches have been proposed to describe the production of viable aerosols from wastewater sources and to predict downwind aerosol concentrations (7, 15, 17, 21, 22). Assuming that the maximum number of live particles remaining in the atmosphere after a certain period of time (t) depends on the physiological sensitivity of the cells and on the atmospheric conditions, and that the biological death (BD) constant (λ) may be determined for a number of specific cases, it was proposed (17) to modify equation 1 to account for λ and t:

$$\chi(x,y,z)_{BD} = \chi(x,y,z)^{\exp(-\lambda t)} \quad (4)$$

where: $\chi(x,y,z)_{BD}$ is the modified concentration considering the microbial die-away rate (particles per cubic meter); t is average aerosol age, in seconds; and λ is microbial die-away constant (per second) as determined experimentally for different microorganisms under various spray irrigation and atmospheric conditions.

If t is approximated by means of x/u, then equation 4 becomes:

$$\chi(x,y,z)_{BD} = \chi(x,y,z)\exp\left(-\frac{\lambda X}{\bar{u}}\right) \quad (5)$$

An additional modification is required when only a part of the material released into the atmosphere becomes an aerosol, as occurs with a sprinkler operating under windy conditions. Thus equation 5 becomes:

$$\chi(x,y,z)_{BD} = \chi(x,y,z)\cdot E\cdot\exp\left(\frac{-\lambda X}{\bar{u}}\right) \quad (6)$$

where E is the aerosolization efficiency factor, i.e., the fraction of the sprayed liquid that actually enters into the atmosphere as an aerosol.

The microbial die-away rate (λ) in the natural atmosphere is a dynamic function of several biological and environmental variables, and to the best of our knowledge it has not yet been measured, except for the work of Camann et al. (7). Laboratory measurements of the microbial die-away rate in air as a function of a number of variables have been performed mostly in steady-state conditions and only rarely in simulation of the natural dynamic environmental conditions (11).

In the following section, a description is given of the experiments performed in our study to

estimate λ under actual dynamic atmospheric conditions and to measure the concentration of bacteria in the environment after their release from a sprayer simulating an irrigation device.

MATERIALS AND METHODS

Experimental site. The site was a flat area situated 750 m above sea level at a distance of 10 km from Jerusalem. The site is a characteristic low shrubland, composed mostly of *Poterium spinosum* bushes up to 50 cm tall.

Sprayer. A Minute Man Jet Fog Sprayer, manufactured by International Industries, Inc., Chicago, Ill., was used. This sprayer produces drops in a diameter range of 18 to 50 μm. The height of the sprayer was 1 m above ground, and the plume was about 1.5 m above ground level. This sprayer was selected because its aerosolization efficiency was presumed to be close to 100%.

Tracer. Since the use of bacteria labeled by radioactive isotopes enables the simultaneous determination of both biological and physical decay, *Escherichia coli* labeled with tritium was used in the present study. *E. coli* was selected because it is a common enteric bacterium and relatively easy to handle.

Cultivation and labeling of bacteria: *E. coli* K-12 cells to be used in the spraying experiments were grown at 37°C in M-9 medium (minimal broth) for one night. In the morning, the bacteria were diluted in the same medium to an optical density of 4 Klett units and then shaken for 2 h at 37°C. L-[4,5-^{3}H]leucine with a specific activity of tritium of 30.3 Ci/mmol (Nuclear Research Center, Negev, Beer Sheva, Israel) was added to this bacterial suspension to achieve a final concentration of 2.10^{-4} mCi/ml, and the suspension was shaken for another hour. The bacteria were then washed in cold medium and centrifuged three times at 10,000 rpm for 15 min each time. The labeled bacteria, packed in ice, were taken to the field in a final volume of 20 ml. The average efficiency of the labeling was $4.7 \pm 3 \times 10^3$ bacteria per cpm; the counting efficiency was 29.6 ± 4.0%, and the background was 39 ± 13 cpm.

Experimental procedures. The labeled bacteria suspension was diluted in 2 liters of distilled water, containing 1,000 μl of beef extract per liter, or 2 liters of sterilized sewage. Spraying time varied between 20 and 60 min. A scrubber-cyclone type sampler (6) was located at a distance of 20 to 40 m downwind from the sprayer, according to the wind conditions and topography of the site. Sampling continued until the completion of spraying. During each experiment a sample (20 ml) of the spray was taken immediately adjacent to the sprayer nozzle. At the end of each experiment a sample (20 ml) of the bacterial suspension was taken from the sprayer container. The collecting fluid of the sampler, of a flow capacity of 3 ml/min, was distilled water containing 1% beef extract. The air capacity was 600 liters/min. The samples were preserved on ice until they were brought to the laboratory, where bacteriological and radioactive measurements were performed on the same day. The net counting rates were in the range of 26 to 11,357 cpm/s.

Meteorological data. The wind velocity in the experimental site was measured by a hand anemometer at the height of 2 m above ground level. The temperature, the relative humidity, and the solar radiation were measured by a meteorological station in Jerusalem. The atmospheric stability classes were determined according to Pasquill's classification (25). All experiments were made during the period from July to September. These months have very little variability in meteorological conditions.

Microbiological and radioactive measurements. The concentration of live bacteria was determined in all samples on Endo agar (Difco) bacteriological plates, as described previously (14). To determine the amount of radioactivity in the spray fluid and in the spray adjacent to the sprayer nozzle, 10 ml of these fluids was first filtered through a 25-mm filter of a pore size of 0.45 μm (Millipore filter HAWPO 2500) and then washed with distilled water and counted by a liquid scintillation counter (Packard) with a toluene-Triton scintillation liquid. The radioactivity in the fluid was determined in the same way, but using the total volume.

Calculation of λ, the microbial die-off constant.
(i) Definitions. In the collecting fluid (sampler): L = concentration of live bacteria (bacteria per milliliter); C = radioactivity concentration (counts per minute per milliliter), total live and dead bacterial count. In the spray adjacent to the sprayer nozzle: L' = concentration of live bacteria (per milliliter); C' = radioactivity concentration (counts per minute per milliliter), total live and dead bacteria count.

(ii) Calculation.

$$R = \frac{L/L'}{C/C'} = \frac{L}{L'} \times \frac{C'}{C} \tag{7}$$

Thus the percentage of surviving bacteria is $R \times 100$, and the percentage of dead bacteria is $(1 - R) = 100$.

Assuming that the biological decay behavior is similar to that of the radioactive decay, $N = N_0 \exp(-\lambda t)$, we may define the microbial die-away constant λ as:

$$\lambda = \frac{\ln(N_0/N)}{t} \tag{8}$$

where N_0 is the ratio between the live bacteria concentration and the concentration of the radioactivity in the spray adjacent to the sprayer nozzle, and N is the ratio between the live bacteria concentration and the radioactivity concentration in the collecting fluid.

By using equation 7 we can rewrite equation 8 as:

$$\lambda = \frac{\ln(N_0/N)}{t} = \frac{\ln(L'/C')(C/L)}{t} = \frac{\ln(1/R)}{t} \tag{9}$$

Lambda (λ) may be calculated from the slope of a graph plotting R as a function of t, assuming a straight-line relationship exists for the major portion of the slope after the rapid reduction in bacterial concentration that occurs in the first 10 to 20 s. This is possible, since $\lambda = |k|$ where k is the slope of the graph.

RESULTS AND DISCUSSION

Sixteen experiments, using distilled water with 1,000 μl of beef extract per liter as spray fluid, were conducted on 8 different days. Eight of these experiments were conducted in the early

morning hours before and at sunrise, with essentially no solar radiation. An additional 14 experiments were conducted with sterilized sewage as the spray fluid, on 8 different days. Six of these experiments were conducted in the early morning hours.

The atmospheric conditions during the morning experiments were different than those encountered during the afternoon experiments. Table 1 shows these conditions.

By assuming the survival factor [exp $(-\lambda t)$] to approach unity (zero decay), the actual efficiency of aerosolization and collection, E, was estimated by the relation between observed $[\chi(x,y,z)_{BD}]$ and predicted $[\chi(x,y,z)]$ aerosol radioactivity strength. Table 2 gives the predicted radioactivity as it was calculated from equation 3 for the emission and sampling at ground level along the centeral axis of the plume and the observed concentration of the radioactivity in air for the series of experiments using sterilized sewage. The mean of E was 1.14 ± 0.52 or an efficiency of 114 ± 54%. The presumed aerosolization efficiency at 100% falls within a standard deviation of the mean and therefore can be accepted as a true assumption.

Figure 1 shows the correlation between the predicted radioactivity and the measured concentrations of the radioactivity in air for the series of experiments using sterilized sewage (r = 0.68). The coefficient of correlation between the predicted and observed radioactivity in air is 0.30 for distilled water with beef extract. However, considering the limitations of the field experiments, the Gaussian plume equations (1 to 3) provide a reasonably good deterioration of the dispersion of the bacterial plume created by the spray, omitting die-away. It should be stressed that due to lack of data, no consideration has been given to the deposition process within the plume path to the sampling site. This process may be important in depleting the plume (23).

When comparing the percentage of live bacteria to the total bacteria in the sprayer reservoir and in the air adjacent to the nozzle, an average loss of 34% occurred, indicating that the act of spraying and aerosolization did not significantly affect the bacterial viability.

Figure 2 shows that no significant differences were noted in the sampler efficiency for diluted or concentrated radioactive aerosols ranging between 10^3 and 10^6 bacteria per m^3 of air. The lowest aerosol concentration sampled was 0.2 cpm/m^3, which is equivalent to 940 bacteria per m^3. The highest concentration was 318 cpm/m^3,

TABLE 2. *Estimation of aerosolization efficiency (spraying with sterilized sewage)*

Time of day	Distance from source (m)	Aerosol density (cpm/m^3)		E
		Observed	Predicted	
05:50	20	13.0	13.0	1.00
05:30	40	1.4	0.6	2.33
06:30	40	0.4	0.2	0.50
05:20	20	3.0	7.0	0.43
05:45	20	18.0	18.0	1.00
13:30	20	3.0	3.0	1.00
14:20	20	3.0	4.3	0.70
13:00	20	3.0	2.2	1.36
14:00	20	1.9	1.7	1.12
12:40	40	5.0	3.0	1.67
13:30	40	1.9	1.3	1.46
17:00	30	11.5	7.3	1.57
18:30	30	4.5	7.0	1.64
Mean ± SD[a]				1.14 ± 0.54

[a] SD, standard deviation.

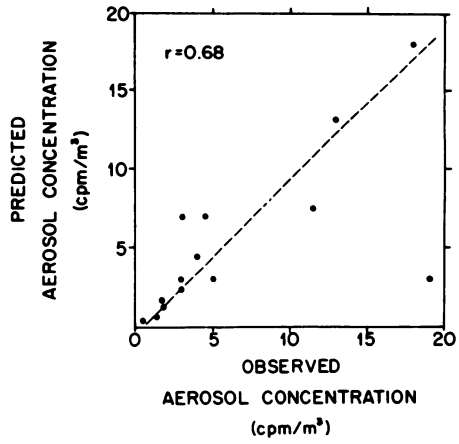

FIG. 1. *Correlation between observed aerosol concentration as counts per minute per cubic meter and predicted concentration according to Pasquill's equation (sterilized sewage).*

TABLE 1. *Atmospheric conditions during the experiments*

Time	Mean wind velocity (m/s)	Mean temp (°C)	Mean relative humidity (%)	Atmospheric stability class	Mean solar radiation (J/cm^2)
Early morning	0.9	17.0 ± 0.6	98 ± 1	B-C	0
Afternoon	2.0	26.5 ± 2.0	50 ± 10	A-B	293.0 ± 41.9

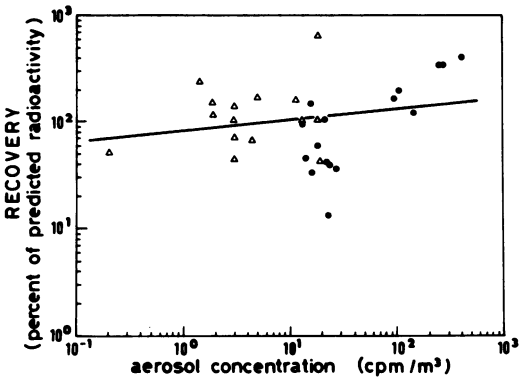

FIG. 2. *Recovery of radioactivity as function of aerosol concentration.* (●) *Distilled water containing 1,000 μl of beef extract per liter;* (△) *sterilized sewage.*

which is equivalent to 1.5×10^7 bacteria per m^3 of air.

As seen in Figures 3 and 4, in the afternoon experiments there was a very rapid die-away rate resulting in a 3- to 4-log cycle reduction in bacterial survival in 30 to 40 s with a 90% reduction (T_{90}) about every 10 s. In the morning experiments, a much slower rate of bacterial die-away was detected with a 1- to 2-log cycle reduction in bacterial survival in about 130 s, with a T_{90} of about 100 s. A sample calculation of R and λ is presented in the appendix.

The bacterial decay rate λ, as calculated from the slope of the regression lines in Fig. 3 and 4, was 9.4×10^{-3} s^{-1} for the morning and 6.4×10^{-2} s^{-1} for the afternoon with distilled water and beef extract, whereas for the series with sterilized sewage, $\lambda = 8.1 \times 10^{-3}$ s^{-1} for the morning and 6.6×10^{-2} s^{-1} for the afternoon. The mean λ for both experimental series was 8.8×10^{-3} s^{-1} for the morning and 6.6×10^{-2} s^{-1} for the afternoon.

The mean λ as calculated according to equation 9, excluding the points during the rapid die-away stage in the first 10 to 20 s, was 2.6×10^{-2} for the morning and 3.4×10^{-1} for the afternoon. Due to limitations in the experimental design, it is felt that the λ calculated by the graph method is the more representative figure.

In general the experiments with distilled water and beef extract as the spray fluid showed less variance than those with sterilized sewage. The correlation coefficient (r) for log R and t for the distilled water series was −0.90 for the morning and −0.68 for the afternoon, whereas with sterilized sewage r was −0.56 and −0.60, respectively. This is based on the assumption that a straight-line relationship exists for the portion of the curve after the first 10 or 20 s.

The results of the experiments indicate that the value of λ, and thus the biological death during the morning hours, was smaller than during the afternoon hours, when the meteorological conditions were more hostile to airborne bacteria. A similar atmospheric influence has been found in previous studies (14, 15).

It may be assumed that the observed decay of a biological aerosol in the atmosphere may result from two major causes: (i) biological decay (e.g., biological inactivation, die-off, and loss of ability to form colonies or plaques under given experimental conditions); or (ii) physical decay (e.g., gravitational settling, mass transfer deposition, and impact on surfaces).

Since atmospheric stability and wind velocity are primarily expected to influence physical dispersion with little known influence on bacterial die-away rates, it can be assumed that with the methods used, which allowed us to disregard physical dispersion, the differences in biological decay are attributable mainly to those environmental factors thought to directly affect bacte-

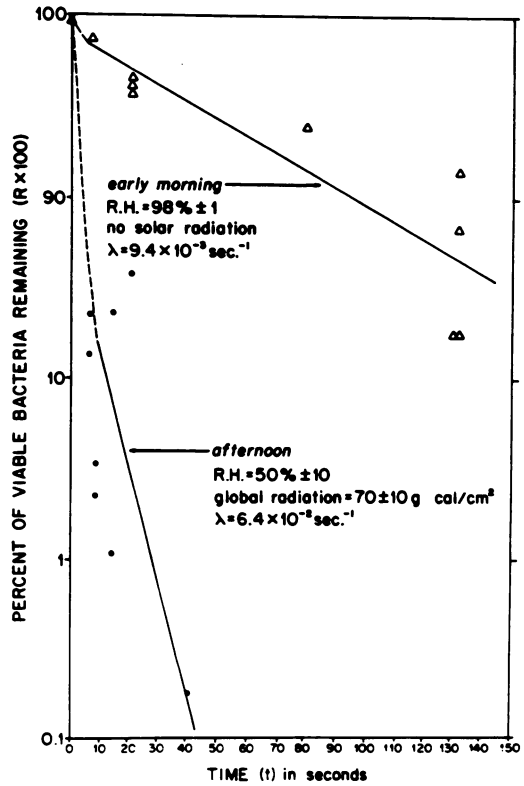

FIG. 3. *Bacterial aerosol reduction as a function of time. Distilled water + 1,000 μl of beef extract per liter. RH, Relative humidity.*

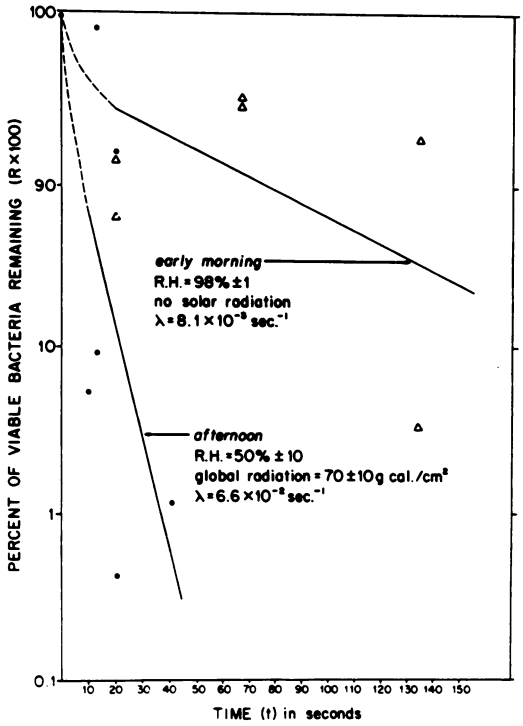

FIG. 4. *Bacterial aerosol reduction as a function of time. Sterilized sewage.*

rial viability, i.e., temperature, solar radiation, and relative humidity.

In our previous field studies using marker bacteria (15) we have shown that temperature in the range between 20 and 27°C had no significant effect on bacterial aerosol viability. Global irradiation in the range of 10 to 80 g cal (ca. 41.9 to 334.9 J) per cm^2 was shown to be weakly negatively correlated with bacterial aerosol survival, and relative humidity between 50 and 85% was shown to have a strong positive correlation with bacterial survival. Based on those previous studies we found that at a relative humidity of about 90% we would expect a 10-times-higher concentration of live bacteria in the air at a given sampling point than at a relative humidity of 50%. This leads us to assume that high relative humidity provides environmental conditions very much more favorable to bacterial survival, which may prove to be the dominant factor in determining the die-away rates of aerosolized bacteria. Solar radiation undoubtedly plays an independent role, particularly under conditions of low relative humidity. It must be remembered that in the early morning experiments radiation was essentially zero, so that it can be assumed that under those conditions and under nighttime wastewater irrigation in general, the relative humidity may provide a possible single parameter capable of predicting, within a given temperature range, the λ required for the model under consideration. Others have also shown the importance of relative humidity in bacterial aerosol survival (10, 11).

The λ determined by the graph method for the afternoon experiments, 6.6×10^{-2} s^{-1}, was undoubtedly influenced by a combination of detrimental environmental factors, including the high solar radiation and low relative humidity, whereas the λ determined in early morning experiments of 8.8×10^{-3} s^{-1} may represent primarily the influence of the high relative humidity (98%) since radiation was zero.

The very rapid die-away of bacteria during the first 10 to 20 s, particularly under afternoon conditions of both low relative humidity and high global radiation, may be associated with the more rapid death of a highly susceptible portion of the bacterial population, or of those appearing as single, more exposed bacteria rather than as bacteria in more protective clumps or embedded in organic particles. Rapid sedimentation of the larger aerosols formed might also contribute to this phenomenon. It can be assumed that the λ varies for these different stages of the decay curve, thus explaining the difference between the calculated figure and that obtained by the graph method on the straight-line portion of the slope. Recognizing the limitations of this experimental design, using only one high-volume sampler in each experiment, we have assumed that the λ by the graph method is the figure that more closely approximates the bacterial die-away rate over long distance.

It is still not possible to use the values of λ determined in this study for practical matters. For example, it cannot be used in predictions of microorganism concentration in the air at a specific downwind distance in an effluent spray-irrigated field.

To further elucidate the questions dealt with in this study, it is essential that λ be determined under a wider range of environmental conditions, at numerous points simultaneously, and for different microorganisms of differing inherent environmental resistance. The goal should be the development and validation of the predictive model for viable aerosolized microorganisms that can be used to aid in the design, siting, and evaluation of wastewater irrigation and wastewater treatment facilities. It is felt that the methodology developed in this study based on the use of tritium-tagged bacterial tracers may help to overcome some of the obstacles that have blocked progress in this area to date.

APPENDIX

Calculation of R and λ. Date: 25 July 1978. $L = 2.5 \times 10^2$ bacteria per ml; $C = 5.4$ cpm/ml; $L' = 1.7 \times 10^5$ bacteria per ml; $C' = 1{,}745$ cpm/ml.

$$R = \frac{L}{L'} \times \frac{C'}{C} = \frac{2.5 \times 10^2 \times 1{,}745}{1.7 \times 10^5 \times 5.4} = 0.475$$

$t = 20$ s.

$$\lambda = \frac{\ln\left(\frac{1}{R}\right)}{t} = 0.038\ s^{-1}$$

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